



# **Cross-amplification of microsatellite loci in *Diploria* reef corals**

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# Corals

- **Base of reef ecosystem**
- **Provide food and shelter**
- **On the decline**
- **Erosion, incr. water temp, sea level rise**
- **Management and conservation**
- **Population structure unknown**

# *Acropora palmata* Study\*

- Elkhorn coral
- Caribbean Sea
- Two genetically different populations



\*Baumns IB, Miller MW, Hellberg ME (2005). Regionally isolated populations of an imperiled Caribbean coral, *Acropora palmata*. *Molecular Ecology* **14**, 1377-1390.





GULF  
OF  
MEXICO

0 MILES 300

ATLANTIC  
OCEAN

CARIBBEAN SEA

PACIFIC  
OCEAN



# *Diploria*

- *D. strigosa* or Brain Coral
  - *D. labyrinthiformis* or Grooved brain coral
  - *D. clivosa* or Knobby brain coral
- Live in clear, low nutrient waters between 1 and 30 m depth
  - Sampled from Florida Keys, Panama, and the Flower Garden Banks in the Gulf of Mexico



D. Labrynthiformas

# Microsatellites

- Tandem repeats of 1-6 nucleotides
- Found at high frequency
- Mutate frequently-  
 $10^{-2} - 10^{-6}$  /locus/generation
- Answer fine-scale ecological questions



# Microsatellite isolation

- Genomics- \$200,000
- Build Species specific microsatellite library- \$10,000
- Cross-species amplification
  - Cost efficient
  - *Favia* and *Diploria* belong to same clad
  - Using m-sat library developed for *F. fragum*\*

\*Carlon, D. B. & Lippè, C. (2008). Fifteen new microsatellite markers for the reef coral *Favia fragum* and a new *Symbiodinium* microsatellite. *Molecular Ecology Resources*, **8**, 870-873.

A blue-tinted image of a human brain, showing the characteristic folds and grooves of the cerebral cortex. The brain is centered in the frame, and the text is overlaid on the left side.

# METHODS

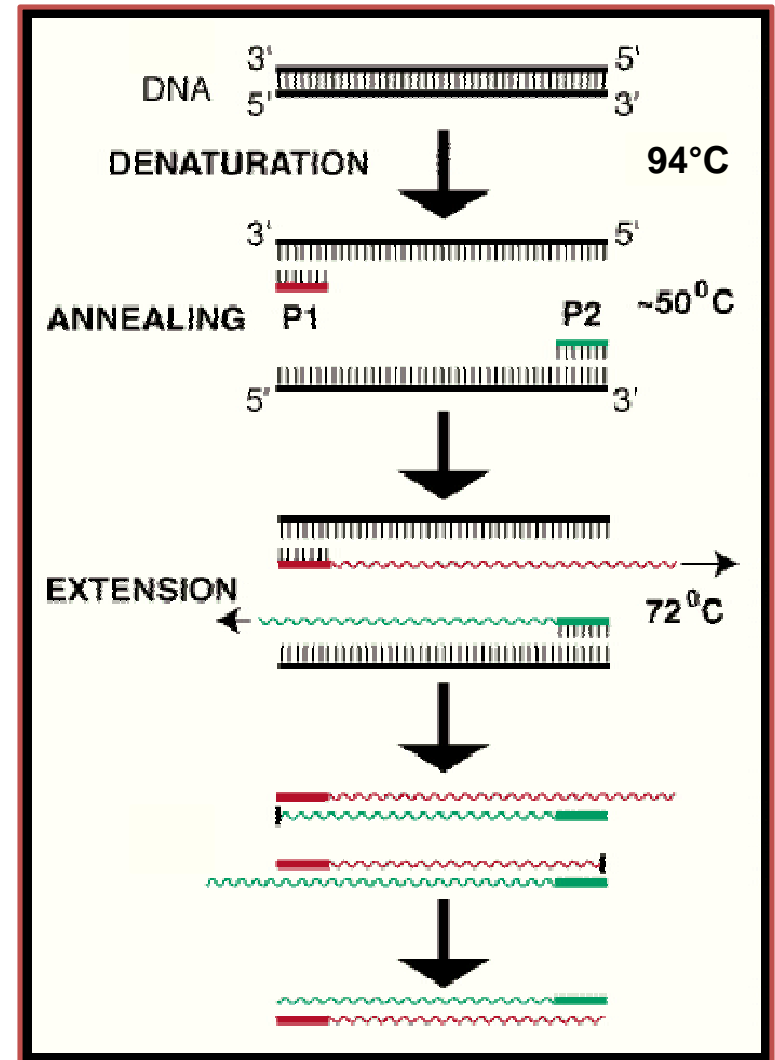
## overview

- DNA Isolation: Qiagen isolation kit
- Polymerase Chain Reaction (PCR)
- Gel Electrophoresis
- Automated Genotyping

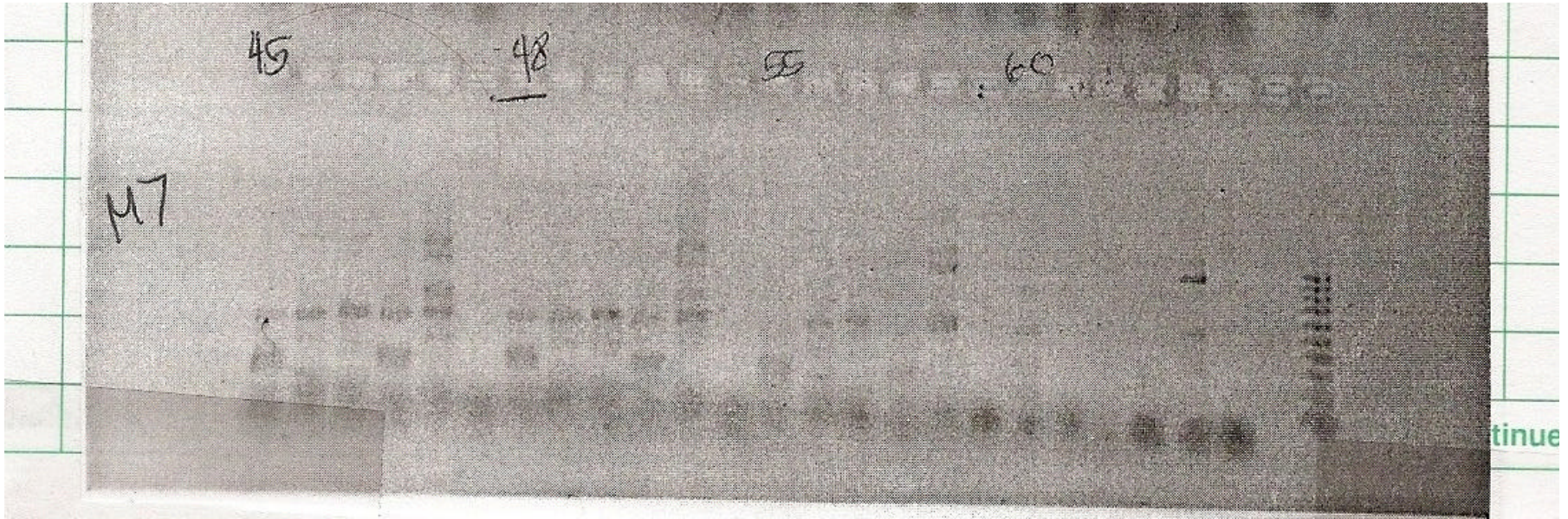


# Gradient PCR

- Cycling Parameters
  - Initial Denature at 94°C for 10min
  - Denature at 94°C for 30sec
  - **Anneal at 45-57°C for 40sec**
  - Extend at 72°C for 60sec
  - Final Extension at 72°C for 10min
  - Cycled 30-32 times
- 11µl reactions
  - 1µl (25-50ng) of total genomic DNA
  - 10X NH<sub>4</sub> reaction buffer
  - 50mM MgCl<sub>2</sub>
  - BSA
  - dNTPs
  - BIOLASE DNA polymerase
  - Forward and reverse primers
  - Water



# Results of gradient PCR



Increase in Temperature? ? ?

1.5% Agarose Gel



# Automated genotyping

- Sent PCR products for automated genotyping
  - ABI 3770 capillary sequencer
  - Snyder hall sequencing facility



# Labeled Primers

Label



Primer



New Strand



Template



Smaller

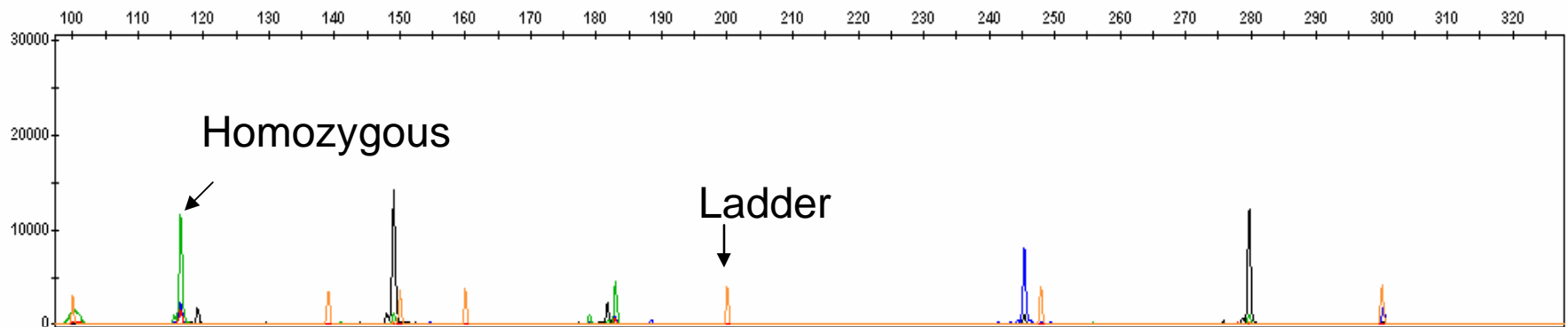


Bigger



# Chromatogram

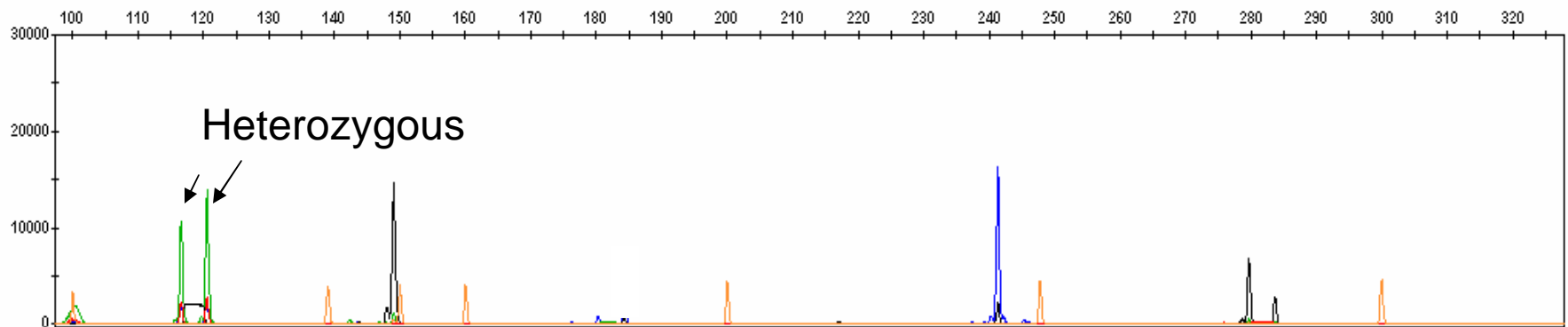
- Scoring chromatogram
  - Ladder
  - Size and intensity



1025-P2\_B04.fsa

1025-P2

None



# Results for 6 of 15 loci

	<i>Diploria clivosa</i>			<i>Diploria labyrinthiformis</i>			<i>Diploria strigosa</i>		
Locus	n <sub>10</sub>	A	Range	n <sub>10</sub>	A	Range	n <sub>10</sub>	A	Range
D7				7	6	117-179			
D10	4	3	143-156	6	3	143-184	3	1	143
B12	4	5	214-321	5	5	252-280	2	3	294-303
Ffr12									
Ffr127									
Ffr31									



Monomorphic



Optimize

A = number of alleles

n<sub>10</sub> = number of samples  
successfully genotyped

Range = size range of alleles



# FUTURE RESEARCH

- Sample size
- Genotyped 30/100 *Diploria* samples
- Cross-amplification of 6/15 microsatellite loci

# APPLICATIONS

- Learn more about population structure including size of population, migration rates and gene flow.
  - May assess future survival rates and historical population sizes
- Management

# Acknowledgements

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- Joanna Bince
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## Thank You

## Questions?

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